Available online at www.sciencedirect.com

SCIENCE DIRECT®

Innovative Food Science and Emerging Technologies 6 (2005) 155–161

Innovative Food Science & Emerging Technologies

www.elsevier.com/locate/ifset

Heat resistance kinetics variation among various isolates of *Escherichia coli*☆

Vijay K. Juneja[a,*], Harry M. Marks[b]

[a]*Food Safety Intervention Technologies Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA*

[b]*U.S. Department of Agriculture, Food Safety Inspection Service, 14th and Independence S.W., Washington, DC 20250, United States*

Accepted 6 March 2004

Abstract

This paper reports an investigation of serotype-specific differences in heat resistance kinetics of clinical and food isolates of *Escherichia coli*. Heat resistance kinetics for 5 serotypes of *E. coli* at 60 °C were estimated in beef gravy using a submerged coil heating apparatus. The observed survival curves were sigmoidal and there were significant differences (p=0.05) of the survival curves among the serotypes. Consequently, a model was developed that accounted for the sigmoidal shape of the survival curves and the serotype effects. Specifically, variance components for serotypes and replicates within serotypes were estimated using mixed effect nonlinear modeling. If it is assumed that the studied serotypes represent a random sample from a population of *E. coli* strains or serotypes, then, from the derived estimates, probability intervals of the expected lethality for random selected serotypes can be computed. For example, expected serotype-specific lethalities at 60 °C for 10 min are estimated to range between 5 and 9 $\log_{10}$ with 95% probability. On the other hand, to obtain a 6-$\log_{10}$ lethality, the expected minutes range, with 95% probability, from 6 to 12 min. The results from this study show that serotypes of *E. coli* display a wide range of heat resistance with nonlinear survival curves.
Published by Elsevier Ltd.

Keywords: Survival curves; Variance components; Nonlinear mixed effects

Industrial relevance: This paper is of high current interest since it deals with the ongoing international debate on log linear vs. non-log linear microbial inactivation curves observed during thermal and non-thermal processing. The data on 5 serotypes of *E. coli* indicate a clear need for further studies with more strains to fully characterize the heat resistance kinetics for *E. coli*.

1. Introduction

Processors use *D*-values to determine necessary time and temperature combinations to obtain specified lethalities to pathogens that might exist in the precooked product. However, the use of *D*-values assumes that the survival curve (log of the number of viable cells versus time) is linear. The consequence of assuming a *D*-value when the survival curve is actually convex is that the degree of safety thought to be achieved may not be so. Thus, it is important for processors to know if survival curves are nonlinear and to take this into account when designing their processing systems.

The nonlinearity of a survival curve is not the only factor that can contribute to designing lethality processes that are not as safe as thought. Often in predictive microbiology lethality experiments are performed using cocktails of different strains, with the purpose of deriving "conservative" estimates of the lethalities that would be obtained for given temperature and time treatments. However, since the number of strains in the cocktail is finite, it is possible that the estimates derived from the cocktail would not be conservative in some circumstances; there may be other strains not studied, or not existing at the time, that would be more or less

☆ Mention of trade names or commercial products in this [article] [publication] is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

* Corresponding author. Tel.: +1 215 233 6500; fax: +1 215 233 6406.
E-mail address: vjuneja@errc.ars.usda.gov (V.K. Juneja).

1466-8564/$ - see front matter. Published by Elsevier Ltd.
doi:10.1016/j.ifset.2004.03.008

heat resistant than those studied. Generally when designing inactivation studies, there is an initial heat resistance screening of many relevant strains (by determining the percentage of surviving cells after a fixed period of time at a given temperature), and the cocktail is made up of the most heat resistance of these strains. Missing in results of experiments using cocktails, however, are the estimates of variability reflecting "between strain" or serotype lethalities. Estimates of between strain variance of lethalities, however, would be useful in constructing safer processes by taking into account the possible variability of the likelihood of cells surviving when assuming that the studied strains represent other strains in some population. In particular, statistical estimates of the range of possibilities (probability intervals) could be made if estimates of the between strain lethality variance were available, assuming that the strains studied represent a random sample of strains for some population and that the distribution of the underlying serotype-specific lethalities is normally distributed.

This paper presents results from inactivation experiments at 60 °C of five different strains of *E. coli*. From these results, survival curves are estimated and the between strain, and between replicate variance components of lethality are estimated. From these estimates probability intervals of obtained lethalities are made assuming an underlying normal distribution.

2. Materials and methods

2.1. Bacterial serotypes

E. coli serotypes isolated from raw processed beef, pork, chicken and turkey, as well as human clinical isolates, were used in the study. The information about these serotypes is given in Table 1. These strains were stored in vials at −70 °C in a mixture (85:15; v/v) of Tryptic Soy Broth (TSB; Difco Laboratories; Detroit, MI) and glycerol (Sigma Chemical, St. Louis, MO).

2.2. Preparation of test cultures

To prepare the cultures, vials were partially thawed at room temperature and 1.0 ml of the culture was transferred to 10 ml of brain heart infusion broth (BHI; Difco) in 50-ml tubes and incubated for 24 h at 37 °C. This culture was not used in inactivation experiments as it contained freeze-damaged cells. A working culture for use in the experiments was prepared by transferring 0.1 ml of each culture to 10 ml of BHI and incubating aerobically for 24 h at 37 °C. These cultures were maintained in BHI for 2 weeks at 4 °C. A new series of cultures was initiated from the frozen stock on a biweekly basis.

Table 1
Escherichia coli sources

Serotype/strain	Isolate designation	Source	Origin
E. coli O157:H7	ent9490 (Jack-in-the-box)	CDC 7	Clinical
E. coli O157:H7	41199-0093	FSIS 9	Deer pepperoni
E. coli	MH98332	FSIS 26	Chicken
E. coli	MH99196	FSIS 32	Cattle
E. coli	MH98355	FSIS 34	Hog

A day before the experiment, the inocula were prepared by again transferring 0.1 ml of each refrigerated culture to 10 ml of BHI, and incubating aerobically for 18 h at 37 °C to provide late stationary phase cells. On the day of the experiment, each culture was centrifuged (5000 Hg, 15 min, 4 °C), the pellet was washed twice in 0.1% peptone water (w/v) and suspended in peptone water to a target level of 10^8–10^9 cfu/ml. The population densities in each cell suspension were enumerated by spiral plating (Model D; Spiral Biotech, Bethesda, MD) appropriate dilutions (in 0.1% peptone water), in duplicate, onto Tryptic soy agar (TSA; Difco) plates and incubating at 37 °C for 48 h.

2.3. Thermal inactivation procedure

The formulation of the model beef gravy used in the present study as a heating menstruum was 1.5% protease peptone, 5.0% beef extract, 0.5% yeast extract and 1.7% soluble starch (Juneja et al., 1998). All ingredients were obtained from Difco. The gravy was sterilized by autoclaving prior to use. Beef gravy (10 ml) was inoculated with 0.1 ml of the diluted inoculum of selected *E. coli* isolates to obtain a final concentration of approximately 10^7–10^8 cfu/ml. Thereafter, the inoculated gravy suspensions were heated at 60 °C using a submerged coil heating apparatus (Cole & Jones, 1990). The submerged coil heating apparatus is comprised of a stainless steel coil fully submerged in a thermostatically-controlled water bath which allows microbial suspensions to be heated between 20 and 90 °C within a short time to achieve a temperature equilibrium. During the heating procedure, samples (0.2 ml) were removed at predetermined time intervals from 5 min to 40 min. Since the lower limit of detection by spiral plating is 21 cfu/ml, 0.6 ml aliquots were removed when low cell numbers were expected at the last 3–4 sampling times. Samples were cooled rapidly in ice slurry.

2.4. Enumeration of surviving bacteria

Decimal serial dilutions were prepared in peptone water and appropriate dilutions were surface plated in duplicate on TSA, supplemented with 0.6% yeast extract and 1% sodium pyruvate, using a spiral plater. Samples not inoculated with *E. coli* were plated as controls. Also, 0.1 and 1.0 ml of undiluted suspension were surface plated, where relevant. All plates were incubated at 30 °C for at least 48 h prior to counting colonies. For each replicate experiment performed in duplicate, an average cfu/g of four platings of each sampling point was used in the statistical analysis.

2.5. Statistical methods

The observed survival curves can be characterized by an initial shoulder and asymptotically approaching a straight line or a convex curve, often referred to as "tailing." While various reasons have been offered that explain tailing (Cerf, 1977), it is assumed here that this occurs due to cell heterogeneity. For fitting these types of survival curves, a function based on the logistic probability distribution has been used in other papers (Augustin, Carlier, & Rozier, 1998; Carlier, Augustin, & Rozier, 1996; Juneja & Marks, 2003)), with ln(t) as the independent variable to model inactivation. The function is

$$\ln(r(t)) = -\ln(1+\exp(a+b\ln(t))) \quad (1)$$

where $r(t)$) is the fraction of surviving cells at time t, and a and b>0 are constants. As t approaches 0 from the right, the limit of the right side is 0 since it is assumed that b>0, and as t approaches infinity, the derivative of the function approaches 0. In other words, asymptotically, the curve approaches a horizontal line, so that the asymptotic D-value is zero. To reflect the possibility of asymptotically non-zero D-values, Eq. (1) can be extended to include the variable time within the exponential function as:

$$\ln(r(t)) = -\ln(1+\exp(a+b\ln(t)+ct)) \quad (2)$$

where a and b>0 and c ∃ 0 are constants. For this function, as t approaches infinity, the derivative approaches $-c$, so that asymptotically, the D-value is ln(10)/c. Thus, a statistical test for a non-zero asymptotic D-value is based on whether c is significantly different from zero. One advantage of Eqs. (1) and (2) that is not shared by some other functions is that, through a transformation, the dependent variable can be expressed as a linear function of the unknown constant values. Eq. (2) can be transformed to

$$\ln\left(r(t)^{-1}-1\right) = a+b\ln(t)+ct. \quad (3)$$

Eq. (3) enables linear regressions to be used to evaluate alternative models and to determine serotype and replicate variance components.

For comparisons to the functions of Eqs. (1) and (2), two other functions were fitted to the data. The derivation of these functions is based on the assumptions that, for survival curves that display tailing, each cell of the population of cells has an asymptotic linear survival curve, described by the parameter k, and that the values of k over the population of cells has distribution $F(k|\theta)$ (Bazin & Prosser, 1988). A function (Juneja, Eblen, & Marks, 2001) which has a shoulder for small times, and then asymptotically converges to a straight line is

$$\ln(p(t|k,w)) = -kt+\ln\left(1+\frac{k}{w}\left(1-e^{-wt}\right)\right) \quad (4)$$

where $p(t)$ is the probability that the cell will be surviving at time t, and k and w are constants. As t 6 4, the derivative of ln($p(t)$) 6 $-k$, and as t 6 0^+, the derivative ln($p(t)$) 6 0 so that Eq. (4) describes a survival curve with an asymptotic D-value and curved "shoulders" with initial slope equal to 0. Assuming w is constant for all cells, and integrating $p(t|k,w)$ with respect to $F(k)$, and taking the logarithm, the survival curve for the population can be described as

$$\ln(r(t)) = \ln(N_F(t))+\ln(1-g_1(t|\theta)f(w)) \quad (5)$$

where $\phi_F(t)$ is the Laplace transform of F, $g_1(t|\theta)$=$\phi N_F(t)$/$\phi_F(t)$, the derivative being taken with respect to t, and $f(w)$=$(1-e^{-wt})/w$, for w>0, and $f(0)$=t. This model is referred to as the nonlinear 1-stage full model. The fourth model considered is the nonlinear spline function:

$$\ln(p(t)) = -b\min(t,t_0)-\ln\left(\phi_F\left((t-t_0)^+\right)\right) \quad (6)$$

where the notation, x^+=max(x, 0) and b, t_0 are values of parameters to be estimated. Bazin and Prosser (1988) suggest, or provide examples of, two distributions for F: a normal distribution, so that ln($\mu_F(t)$)=$-:t+(vt)^2/2$, where $:$ is the mean and v is the standard deviation of F; and a gamma distribution, so that ln($\mu_F(t)$)=$-(:/v)^2$ ln$(1+v^2t/:)$, where again $:$ is the mean and v is the standard deviation of F. However, for large t the normal distribution implies an increasing survival curve, which we assume is not possible. Thus, the gamma distribution is assumed in this paper.

Table 2
Root-mean-square errors (RMSE) for different models of survival curves for each replicate and serotype

Serotype	Rep	RMSE logit (2 parameters)	RMSE logit (3 parameters)	RMSE one stage (3 parameters)	RMSE spline gamma (4 parameters)
A	1	0.408	0.172	0.128	0.172
A	2	0.278	0.300	0.354	0.394
A	3	0.349	0.331	0.299	0.327
B	1	0.673	0.690	0.703	0.684
B	2	0.184	0.195	0.483	0.222
C	1	0.176	0.121	0.656	0.112
C	2	0.310	0.334	0.564	0.342
D	1	0.387	0.405	0.459	0.313
D	2	0.352	0.380	0.905	0.535
E	1	0.371	0.244	0.617	0.130
E	2	0.410	0.358	0.337	0.089
Pooled	RMSE	0.376	0.352	0.542	0.350

For determining the values of the parameters of the nonlinear regression, for each serotype and replicate, ordinary least squares (OLS) nonlinear regression routines were used from SAS®-PC release 8.00, PROC NLIN. For computing root-mean-square errors (RMSE) for the models, the sum of squares of the residuals was divided by $n-1-p$, where n is the number of data observations, p is the number of parameters in the model, and the -1 reflects that the origin (time=0) was not used in the regression.

Nonlinear and linear mixed effects models were fit to determine variance components associated with the serotype and replicate effects. These analyses were performed on S-plus -6.0, professional, release 1, using the nonlinear mixed effects procedure, and PC-SAS, using the PROC MIXED procedure (Pinheiro & Bates, 2000). However, the idea of these types of analyses is to determine the variance components associated with the parameters of the model that arise due to the correlations that exists among the observations of experiments. In our case, correlations exist because the results are either within the same experiment (replicate) or of the same serotype. Hence the variance structure is nested, consisting of two levels of variance components: (1) serotype, and (2) the replicate within serotype. So, if $\forall$ is a random parameter, then it is assumed that $\forall$ has an expected value, $\therefore$, over some population of serotypes and a standard deviation, Φ_S, reflecting the between serotype variability. Furthermore, over a series of experiments for a given serotype, there would be a standard deviation, Φ_e of the observed or measured results of $\forall$. Hence, for a (infinite) series of measured values, a_j, of $\forall$, where for each experiment a randomly chosen serotype is used, the expected mean value of the value of a_j's is $\therefore$, and the expected variance of the a_j's is, $\Phi_S^2+\Phi_e^2$. The notion is extended for more than one parameter, where it is assumed that the parameters' values are correlated. To clarify the nomenclature: when there is no random component for a parameter, the parameter is referred to as a fixed parameter; otherwise the parameter is a random parameter. A random parameter induces more (fixed) parameters because of the associated variance components and correlations. Thus, even a small number of random parameters that are initially designated in a model can result in a rather large number of fixed parameters that need to be considered. For example, if there are three random parameters, then, for the variance structure described above, there are a total of 15 fixed parameters: 3 associated with the expected values; plus 12 associated with the variances and covariances for the two levels of variance components. When all possible fixed parameters are included in a model, then the model is referred to as being full. Because of the large number of fixed parameters, convergence is (often) not obtained, particularly when there are small numbers of serotypes and replicates. Thus simplifying assumptions are made with the goal of reducing the number of parameters that are needed to describe the model well.

To determine a model that incorporates serotype and replicate variance components various choices of independent variables and assumed variance structure are tried with the goal of finding a set which provides a relatively good fit, using the likelihood ratio test. For example, for determining if p added fixed parameters to the model improve the fit significantly, the likelihood ratio test statistic (the change of -2 log-likelihood when adding the parameters) is compared to the percentiles of the chi-square distribution with p degree of freedom. For example, if p=1, if the likelihood ratio test statistic is greater than 6.64, then the added term is significant at the 0.01 level. Besides the likelihood ratio test, an examination of the pattern of residuals and the

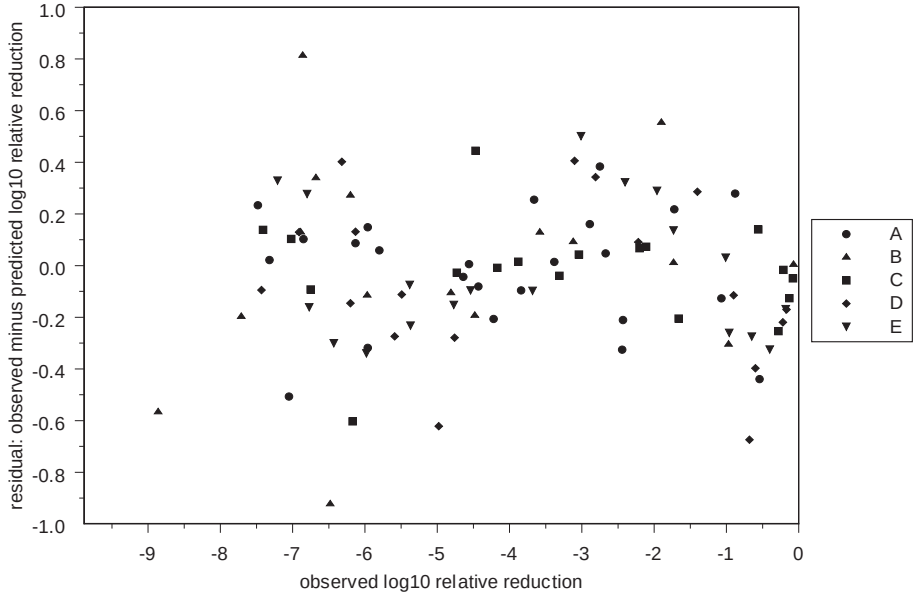

Fig. 1. Residuals: observed minus predicted $\log_{10}$ relative reduction obtained from nonlinear regressions of Eq. (2) for each experiment at 60 °C for 5 serotypes of *E. coli*., versus the observed $\log_{10}$ relative reduction. Symbols represent the different serotypes studied.

significance of the values of the estimated values of the parameters are also considered.

3. Results and discussion

Following usual practice, the above equations were transformed to $\log_{10}$ units. The regressions were performed with the $\log_{10}$ of the ratio of the observed levels (cfu/ml) to the levels at time 0 as the dependent variable. Table 2 presents the root-mean-square errors (RMSE) for the 4 different nonlinear regression models discussed above: the logistic with 2 parameters (Eq. (1)); the logistic with 3 parameters (Eq. (2)); 1-stage full model, using the gamma function (Eq. (5)) and the spline function (Eq. (6)). The 3 parameter–logistic and the spline functions had the lowest pooled root-mean-square errors of about 0.35 $\log_{10}$. Because the logistic function with 3 parameters provides good fits to the observed observations, as well as the spline function that uses 4 parameters, the logistic function (Eq. (2)) is used to further develop a model of inactivation for *E. coli* at 60 °C. Fig. 1 is a plot of the residuals from the nonlinear regressions of Eq. (2) versus the observed $\log_{10}$ relative reductions. There are not significant patterns of residuals in this figure; for the most part, the spread of the residuals is nearly homogeneous. The standard deviation of the residuals is 0.284 and the Shapiro–Wilks test for normality had a p-value of 0.65. For comparison, the standard deviation for the residual using the nonlinear regression of Eq. (1) is 0.324, which is, as expected slightly larger, and the Shapiro–Wilks test for normality had a p-value of 0.13, primarily due to an asymmetric distribution of the residuals.

Table 3 provides further detail of the estimated values of the parameters of Eq. (2). This table presents the estimated values of a, b, and c, the asymptotic D-values, the $\log_{10}$ of the asymptotic D-values, the standard error of the asymptotic D-values, and the one-sided significance value for testing $c>0$. For all but two of the 11 regressions, the estimated value of c is positive and most of these are significant at better than the 0.10 one-sided level. However, as is evident, there is a great amount of variability among the results of the individual experiments.

Table 4
Nonlinear models (Eq. (2)) and log-likelihood values

	Variance components		Number of	
	Serotype	Replicate	Parameters	Log-likelihood
1	(a, b, c)	(a, c)	13	−59.72
2	(a, b)	(a, c)	10	−60.74[a]
3	(a, b)	(b, c)	10	−62.99
4	(b)	(a, b, c)	11	−60.95
5	(a, b; corr=0)	(a, c)	9	−61.22
6	(b)	(a, c)	8	−61.23[a]
7	( )	(a, c)	7	−135.65

[a] P-value for testing significance of model with more parameters=0.61.

One of the objectives of this paper is to determine the variance components associated with the values of the heat inactivation parameters. To designate mixed effect models that were tried the following notation is used: if x is a random parameter assumed to have variance component at level s, then s will be designated as a function of x, $s(x)$. For example, a model that assumed that b varies by serotype, and a and c vary by replicate within serotype, would be designated as Serotype(b) and Rep(a, c).

Mixed effect regressions were performed, using Eq. (2) for various assumed variance matrices. Convergence was not obtained for the full models (assuming the possibility of all possible non-zero correlations) for both linear and nonlinear models. The models overestimated the lethalities obtained for the shortest time (=1 min) greater than zero for which measurements were made. While most of the observed lethalities at this time were small, averaging about 0.5 $\log_{10}$, and all were less than about 1 $\log_{10}$, the fitted curves were flat for small times, resulting in negative residuals. Since the predictions are desired for larger lethalities, these data points were deleted from the subsequent analysis. Thus, the developed model applies for times greater than 1 min. Table 4 gives a list of some of the models that were analyzed. In the table, the number of parameters and the log-likelihood values are provided. Other models not shown had associated lower likelihood

Table 3
Estimated parameter values for logistic regression: $\log_{10}(r(t))=-\log_{10}(1+e^{a+b\ln(t)+ct})$, where $r(t)$ is the relative reduction of viable *E. coli* at time t

Serotype	Rep	a	b	c	Asymptotic D-value (min)	Log10 asymptotic D-value	Standard error asym. D-value	One-sided P-value for $c>0$
A	1	1.09	2.07	0.96	2.39	0.38	0.42	0.002
A	2	−1.83	4.99	0.48	4.75	0.68	4.26	0.163
A	3	0.70	4.71	0.45	5.15	0.71	3.47	0.090
B	1	0.46	5.10	0.83	2.79	0.45	2.32	0.142
B	2	−1.89	7.89	0.21	11.16	1.05	16.95	0.270
C	1	−6.62	0.62	1.79	1.29	0.11	0.30	0.003
C	2	−10.58	11.25	0.00			0.00	
D	1	−4.82	7.91	0.48	4.79	0.68	6.65	0.249
D	2	−17.25	15.23	0.00	–	–	0.00	–
E	1	−5.63	0.00	1.98	1.16	0.06	0.06	0.000
E	2	−2.40	3.05	0.84	2.75	0.44	0.86	0.008

Also included is the estimated asymptotic D-value, standard error of this, and the one-sided significant value for $c>0$.

values (more negative) or did not show improvement versus the ones shown. The best model shown in this table has the between serotype variance components being a function of the variable b: Serotype(b), Rep(a,c). The estimated values, with standard errors in parentheses, of the fixed parameters for that model are: $E(a)=-4.41$ (1.30); $E(b)=5.94$ (0.95); $E(c)=0.679$ (0.178). The standard deviation of the residuals is 0.34. From the expected value of c, the expected asymptotic D-value is estimated as: $\ln(10)/E(c)=3.39$ (0.89) min. The estimated standard deviation, S.D., for the between serotype level is S.D.(b)=0.984, and for the between replicate level, the standard deviations of a and c and the correlation between them are: S.D.(a)=3.75, cor(a,c)=−0.974, S.D.(c)=0.303.

Fig. 2 presents the fitted survival curve, using the above model, together with curves surrounding the fitted curves giving various probability intervals of expected lethalities. To explain the curves imagine an experiment consisting of an infinite number of trials where for each trial a serotype is chosen at random from a large (but possible finite) population of serotypes and a survival curve for that trial is determined (with virtually no error). For each serotype, an average curve is obtained, which is referred to as the serotype-specific average survival curve. The fitted curve represents an estimate of the average of all the trial-specific survival curves, or the average of all the serotype-specific average survival curves; the two curves that are closest to the fitted curve represent estimates of the boundaries of a region for which 95% of the serotype-specific average curves fall within; the outer most two curves represent a region for which 95% of the trial-specific survival curves fall within. For example, to obtain a 6-$\log_{10}$ lethality, the expected serotype-specific minutes range, with 95% probability, from 6 to 12.2 min. On the other hand the expected serotype-specific lethality at 60 °C for 10 min is estimated to range between about 5 and 8.9 $\log_{10}$ with 95% probability. A referee mentioned a recent paper (Whiting & Golden, 2002) that reported D-values for 17 strains of *E. coli* O157:H7 cooked in brain heart infusion broth at 60 °C (as well as 55 °C). Using the estimated D-values at 60 °C, estimated lethalities for 10 min of cooking ranged from 4.7 $\log_{10}$ to 14.5 $\log_{10}$. The length of this range is larger than that of the 95% probability interval given above. Differences in the study designs (broth and strains studied) of the two papers and inherent statistical variation certainly can contribute to the differences of estimated lethalities from the two papers. In addition, though, these differences, in part, could be due to features of the model used: (1) the reported range of the 17 strains includes the effect of within-strain, between-replicate variability, while the 95% probability interval estimated from the model of this paper, theoretically, does not; (2) some of the survival curves for the 17 strains could be convex for large times, for which lethality predictions, based on estimated D-values, which do not account for the convexity, would overestimate the actual lethality; (3) some of the survival curves for the 17 strains could have had shoulders and very little, or no, convexity for large time, for which lethality predictions, based on estimated D-values that do not account for the shoulder would underestimate the actual lethality. In support of the

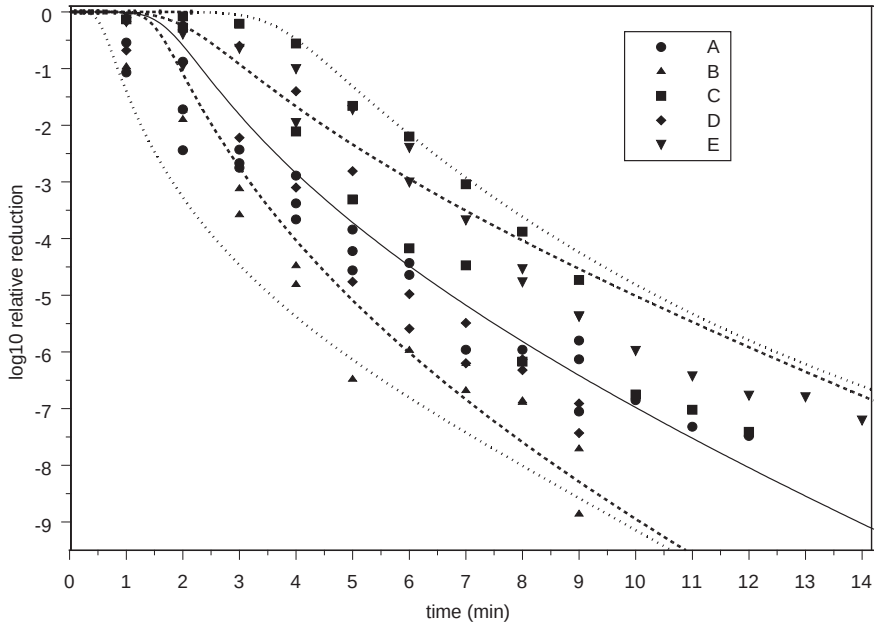

Fig. 2. Fitted survival curve (center curve) using mixed effect nonlinear model. The two curves closest to the center curve (- - -) represents the estimated upper and lower boundaries for which 95% of the serotype-specific survival curves would fall within; the two outer curves (· · ·) represent the estimates of the upper and lower boundaries for which 95% of the trial or experimental-specific survival curves would fall within. Also included are the observed $\log_{10}$ relative reductions.

second in the above list of possible reasons, convex shaped graphs of observed survival curves for 6 *E. coli* O157:H7 strains heated at 52 °C and 57 °C were shown in a paper (Benito, Ventoura, Casedei, Robinson, & Mackey, 1999) also suggested to us for consideration by a referee.

4. Conclusion

Estimates of survival curves at 60 °C for 5 serotypes of *E. coli* were examined. The estimated survival curves were sigmoidally shaped, but tending, asymptotically, to a nonzero *D*-value. In support of this result, in another paper (Benito et al., 1999) convex shaped survival curves were displayed. In addition, the estimated survival curves for the different serotypes were significantly different.

Within the paper an expected survival curve was derived together with between serotype variance components of parameter values used for describing the survival curves. The function used to describe the survival curve is based on the logistic function: $-\log_{10}(1+\exp(a+b\ln(t)+ct)$, where t is time, and a, b, and c are parameters. The asymptotic D-value is $\ln(10)/c$. From the estimates, probability regions describing the range of expected serotype-specific survival curves can be computed. A 95% probability interval for the time needed to obtain a 6-$\log_{10}$ lethality at 60 °C was estimated at 6 to 12 min. In other words, it was estimated that, for 2.5% of the *E. coli* serotypes, more than 12 min is needed to obtain an expected 6 $\log_{10}$ lethality. The results of the study, based on 5 stereotypes of *E. coli*, indicate that nonlinearity and potential significance between serotype variations in heat resistance exist for *E. coli*. Consequently additional study with more strains is needed to fully characterize the heat resistance kinetics for *E coli*.

Acknowledgments

We thank Ms. Angie Osoria for technical assistance, and Drs. John Phillips, Rolando Flores, and Lihan Huang of the Agricultural Research Service, who reviewed and made helpful comments which led, we think, to substantial improvements in the paper. We also thank the referees who reviewed the paper and provided additional references.

References

Augustin, J. C., Carlier, V., & Rozier, J. (1998). Mathematical modelling of the heat resistance of *Listeria monocytogenes*. *Journal of Applied Microbiology*, *84*, 185–191.

Bazin, M. J., & Prosser, J. (1988). Physiological Models in Microbiology II, *CRC Series in Mathematical Models in Microbiology*. Boca Raton, FL: CRC Press, Inc.

Benito, A., Ventoura, G., Casedei, M., Robinson, T., & Mackey, B. (1999). Variation in resistance of natural isolates of *Escherichia coli* O157 to high hydrostatic pressure, mild heat, and other stresses. *Applied and Environmental*, 1564–1569.

Carlier, V., Augustin, J. C., & Rozier, J. (1996). Heat resistance of *Listeria monocytogenes* (Phagovar 2389/2425/3274/2671/47/108/340): *D*- and *z*-values in ham. *Journal of Food Protection*, *59*, 588–591.

Cerf, O. (1977). Tailing of survival curves. *Journal of Applied Bacteriology*, *42*, 1–19.

Cole, M. B., & Jones, M. V. (1990). A submerged-coil heating apparatus for investigating the thermal inactivation of bacteria. *Applied Microbiology in Letters*, *11*, 233–235.

Juneja, V. K., Klein, P. G., & Marmer, B. S. (1998). Heat shock and thermotolerance of *Escherichia colia* 0157:H7 in a model beef gravy system and ground beef. *Journal of Applied Microbiology*, *84*, 677–684.

Juneja, V. K., Eblen, B. S., & Marks, H. M. (2001). Modeling non-linear survival curves to calculate thermal inactivation of Salmonella in poultry of different fat levels. *International Journal Food Microbiology*, *70*, 37–51.

Juneja, V. K., & Marks, H. M. (2003). Mathematical description of non-linear survival curves of *Listeria monocytogenes*. *Innovative Food Science and Emerging Technologies*, *4*, 307–317.

Pinheiro, J. C., & Bates, D. B. (2000). *Mixed-effects Models in S and S-plus*. New York: Springer PC-SAS®. 1999.

Whiting, R. C., & Golden, M. H. (2002). Variation among *Escherichia coli* O157:H7 strains relative to their growth, survival, and thermal inactivation, and toxin production in broth. *International Journal of Food Microbiology*, (75), 127–133.